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A genome scan for loci influencing total serum immunoglobulin levels: possible linkage of IgA to the chromosome 13 atopy locus

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Immunoglobulins play an essential part in the immune system, and immunoglobulin deficiencies can have profound medical consequences. The genetic control and regulation of the immunoglobulin response is therefore of interest. Previous investigations have identified a number of loci influencing total and specific IgE levels. In this study, 80 nuclear families have been examined for linkage of total serum IgA, IgG and IgM levels to a genome-wide panel of microsatellite markers. Potential quantitative trait loci influencing IgA levels have been identified on chromosomes 10 and 13, and possible loci influencing IgG levels were found on chromosomes 3 and 13. No significant linkages to IgM levels were found. The linkage of IgA on chromosome 13 was to a marker previously linked to IgE responses (atopy). Linkage to IgG was in the same region but to a more distal marker. None of the factors known to influence immunoglobulin expression map to the loci identified in the present study. These loci are therefore likely to contain previously unrecognized components of the immunoregulatory system.

INTRODUCTION

Immunoglobulins play an essential part in the immune system. There are five classes (or isotypes) of immunoglobulin: IgA, IgD, IgE, IgG and IgM. Each isotype, except for IgD, has a known biological role. Congenital and acquired immunoglobulin deficiencies have profound medical consequences (1,2), and there is interest in identifying factors involved in the control of the immunoglobulin response. Cellular and molecular studies have characterized the roles of many cytokines in the control and regulation of immunoglobulin production (3). Genetic linkage and positional cloning may also be used to detect genes that regulate immunoglobulin production.

Genetic studies have focused on IgE (4). Elevated levels of IgE are associated with the syndrome of atopy (familial asthma, rhinitis and eczema). The atopic state may be identified clinically by a combination of skin prick tests to common allergens, specific serum IgE titres to these allergens and raised total serum IgE levels. This combination has been used to describe atopy phenotypically for genetic studies (5).

Linkage analysis of 80 nuclear families with a genome-wide panel of markers has identified quantitative trait loci influencing total serum IgE levels on chromosomes 11 and 16 and a locus linked to atopy on chromosome 13 (5). Other authors have found possible loci influencing total serum IgE levels on chromosome 13 (6), chromosome 5 (7,8) and chromosome 12 (9). The atopy locus identified by Daniels and Bhattacharyya *et al.* (5) is close to, and likely to be the same as, the IgE locus on chromosome 13 (6).

Previous family studies have suggested heritable components to the serum levels of IgA, IgG and IgM (10–12). In order to investigate further the genetic control of the antibody response, we have examined the familial correlations and heritability of these traits in 232 families from a random population sample. We have then searched for linkage between total serum IgA, IgG and IgM levels and a genome-wide panel of microsatellite markers in a sub-sample of 80 nuclear families. These families have been examined previously for linkage to quantitative traits underlying asthma and atopy (5).

RESULTS

Preliminary data analysis

Descriptive statistics for total serum immunoglobulin levels in the whole sample are summarized in Tables 1 and 2. Immunoglobulin phenotypes for 455 parents and 536 offspring were available. Sons and daughters were equally represented. There were 203 offspring in the sample of families used for the genome screen, and these formed 172 sib pairs. The mean levels (± standard error) of IgA, IgG and IgM in the genotyped families were 1.49 ± 0.041, 10.13 ± 0.15 and 1.24 ± 0.04g/l, respectively.

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Table 1. Descriptive statistics for total serum IgA, IgG and IgM levels of parents in the whole sample

	IgA	IgG	IgM
Range of untransformed Ig level	0.08–4.66 g/l	3.53–18.3 g/l	0.31–9.80 g/l
Mean of untransformed Ig level (±SE)	2.14 ± 0.04 g/l	10.80 ± 0.10 g/l	1.50 ± 0.04 g/l
Mean of transformed Ig level	1.43	3.27	0.29

Ig, immunoglobulin; SE, standard error.

Table 2. Descriptive statistics for total serum IgA, IgG and IgM levels of offspring in the whole sample

	IgA	IgG	IgM
Range of untransformed Ig level	0.07–4.34 g/l	3.85–16.2 g/l	0.31–3.58 g/l
Mean of untransformed Ig level (±SE)	1.48 ± 0.03 g/l	9.83 ± 0.09 g/l	1.20 ± 0.02 g/l
Mean of transformed Ig level	1.18	3.12	0.09

Ig, immunoglobulin; SE, standard error.

Table 3. Familial correlations of adjusted IgA, IgG and IgM in the whole sample

Immunoglobulin	Familial correlation			
	Father–mother	Mother–offspring	Father–offspring	Offspring–offspring
IgA	0.085	0.362	0.359	0.379
IgG	0.185	0.320	0.346	0.414
IgM	0.032	0.401	0.338	0.452

IgA and IgG levels in the whole population were transformed with a square root. IgM levels were transformed with a natural logarithm. Transformed levels were very close to normality. Levels of IgA, IgG and IgM increased significantly with age in sons and daughters ($P < 0.0005$). There was no significant effect of smoking on any immunoglobulin following adjustment for age in offspring. Slight adjustments for age on mothers' IgG levels and for smoking on fathers' IgG levels were necessary. Adjusted levels were standardized in each family group.

Familial correlations

The results for familial correlations for adjusted IgA, IgG and IgM in the whole sample are shown in Table 3.

Father–mother correlations were not significantly different from zero for IgA levels ($\chi^2 = 1.68$, $P = 0.19$) and IgM levels ($\chi^2 = 0.24$, $P = 0.63$). The father–mother correlation for IgG levels did differ significantly from zero ($\chi^2 = 7.91$, $P = 0.005$). Absence of mother–offspring, father–offspring and offspring–offspring correlations was rejected for all three phenotypes ($P << 0.0001$). Mother–offspring and father–offspring correlations did not differ significantly from one another for IgA ($\chi^2 = 0.003$, $P = 0.96$), IgG ($\chi^2 = 0.20$, $P = 0.66$) or IgM ($\chi^2 = 1.11$, $P = 0.29$).

Heritability calculations

The estimates for the genetic heritability of unadjusted transformed IgA, IgG and IgM levels from the whole sample are shown in Table 4.

Table 4. Heritability estimates of unadjusted immunoglobulins A, G and M in the whole sample

Immunoglobulin	Heritability
IgA	69.2%
IgG	61.8%
IgM	60.4%

Linkage analysis

The results of the genome-wide linkage study are summarized in Table 5. Marker order is that determined by Daniels *et al.* (5), and is consistent with the published CEPH order (13). Genetic distances are calculated from recombination fractions determined using the LINKAGE program (14).

There was evidence for linkage of IgA to marker *D10S190* on chromosome 10 ($P = 0.00004$) and marker *D13S153* on chromosome 13 ($P = 0.0009$). There was a suggestion of linkage of IgA to marker *D3S1300* on chromosome 3 ($P = 0.004$).

There was evidence for linkage between IgG and *D13S120* on chromosome 13 ($P = 0.0008$). The recombination fraction between *D13S120* and *D13S153* (which showed linkage to IgA) was 0.21. There was a suggestion of linkage between IgG and a marker on chromosome 3 ($P = 0.001$) and on chromosome 16 ($P = 0.002$). There was no linkage between IgA, IgG or IgM and any marker on the X chromosome.

The significance levels of these linkages were not significantly changed by removing sibs contributing to outlying values of the squared sib-pair difference greater than three standard deviations from the mean.

Table 5. Results of sib-pair linkage analysis with the genome-wide marker screen

Marker	Genetic distance (cM)	IgA	IgG	IgM
D3S1263	44.6	n.s.	0.001	0.03
D3S1300		0.004	n.s.	n.s.
D10S190	–	0.00004	n.s.	n.s.
D13S192	3.8	n.s.	0.007	n.s.
D13S120	22.1	0.002	0.0008	n.s.
D13S153	4.6	0.0009	0.03	n.s.
D13S262		**n.s.**	**n.s.**	**n.s.**
D16S265	3.0	n.s.	0.002	n.s.
D16S503		0.02	0.03	n.s.

Figures are *P*-values, given to one significant digit. Only regions containing linkages to one or more phenotypes with *P*-values <0.005 are shown, together with linkages to flanking markers. These results were calculated using unambiguous genotypes and therefore were not dependent on allele frequencies for the calculation of allele sharing ratios. Each genetic distance (cM) is to the subsequent marker and is calculated using the Kosambi map function from the corresponding recombination fraction. n.s.: non-significant at the 5% level.

DISCUSSION

Heritability estimates and familial correlations for IgA, IgG and IgM in the whole sample suggest a strong heritable component to serum immunoglobulin levels. This is in general agreement with previous findings, although the estimates from the present study are higher than those previously reported (10–12). These findings are encouraging for the search for quantitative trait loci influencing immunoglobulin levels. The non-zero father–mother correlation for IgG suggests a significant environmental component to serum IgG levels and that consequently the heritability estimate of IgG might be inflated.

This study provides linkage evidence for quantitative trait loci influencing levels of total serum IgA on chromosomes 10 and 13. There is a suggestion of linkage of IgA to chromosome 3. A quantitative trait locus influencing total serum IgG levels was found on chromosome 13, together with possible linkages on chromosomes 3 and 16. The nominal significance level associated with a LOD score of 3, the classical criterion for declaring significant linkage, is ~0.0001, but varies, with an upper value of 0.001 (15,16). The linkages on chromosome 13 meet this upper limit, and that on chromosome 10 conforms to the more stringent value.

The linkage of IgA on chromosome 13 is to the same marker (*D13S153*) as that showing linkage to atopy in the previous study of the same set of families (5). The linkage of IgG on chromosome 13 is to a different marker (*D13S120*) that is 22 cM from *D13S153*. The results are consistent with a gene linked to *D13S153* that influences both the atopic state and total serum IgA levels.

Low levels of serum IgA are more frequent than expected in young atopic patients (17), and transient deficiency of serum IgA is associated with an increased incidence of atopy in infants (18). Infants with serum IgA levels in the lowest quartile of the normal range have a higher prevalence of atopy than other children (19). Salivary IgA deficiency is more common in infants born to atopic parents than those with non-atopic parents (20). However, not all IgA-deficient patients are atopic (20).

IgA deficiency may allow increased antigen penetration to the mucosa, with subsequent development of an exaggerated IgE response (18). Alternatively, there may be a defect in a regulatory component common to IgA and IgE production rather than a secondary link between IgA deficiency and atopy (20). The common linkage of IgA and atopy on chromosome 13 supports this hypothesis. The linkage of IgA on chromosome 10, which is not shared with IgE, also suggests the presence of independent components of IgA regulation.

The marginal significance levels of the results for chromosomes 3 and 16 require that these regions be investigated further to establish whether they are genuine linkages. If so, the linkages of IgA and IgG on chromosome 3, being ~45 cM apart, are likely to be two distinct loci. Similarly, the possible linkage of IgG to chromosome 16 is in a different region from that shown to contain a linkage to IgE in the previous study of the same set of families (5).

This study provides no linkage evidence for quantitative trait loci influencing the levels of serum IgM, despite the presence of significant parent–offspring correlations and genetic heritability. Linkage analysis has little power to detect genes with small effects (21). It is possible that serum IgM levels are under the control of a larger number of loci having individually small effects, or that the number of families and marker density in the genome screen were insufficient to detect genes of greater effect.

Immunoglobulin production is known to be under the control of cytokines such as interleukins 4, 5, 10 and 13, γ-interferon and transforming growth factor-β (3). None of these cytokines maps to the loci identified in the present study. The loci on chromosomes 10 and 13 are therefore likely to contain previously unrecognized components of the immunoregulatory system.

The true significance of results from genome-wide linkage studies of complex diseases is difficult to assess (22–25). Although simulation-based methods may establish the global significance of results from such studies (26), replication of positive results in additional families is required for the loci identified in the present investigation.

MATERIALS AND METHODS

Pedigrees

The whole population sample comprised 232 families randomly ascertained from the town of Busselton, Western Australia. These families contained 1020 individuals. Parents' ages ranged from 27 to 55 years and offsprings' ages ranged from 5 to 27 years. The subset of this larger sample used for the genome screen contained 80 nuclear families selected on the basis of their atopic status (5). The subset contained 203 offspring forming 172 sib-pairs.

Phenotypes

Total serum IgA, IgG and IgM levels were measured from single blood samples taken during the winter months from the whole sample. Rate nephelometry with a Beckman array calibrated against the most recent international protein standard (CRM470) was used. Serum IgD levels were not determined. Atopy was defined phenotypically as a combination of one or more of the following: the sum of the skin-prick test responses to house dust mite and grass pollen ≥5 mm; the sum of the RAST score to house dust mite and timothy grass ≥2; and a total serum IgE level greater than the 70th percentile of the age- and sex-corrected population (5).

Genotypes

Genotyping was carried out as previously described (5). Total genomic DNA was extracted from peripheral blood leucocytes and genotyped with 265 microsatellite markers (253 autosomal and 12 X chromosome) using the fluorescence-based semi-automated method of Reed *et al.* (27). These markers had an average heterozygosity of 75% based on the families in the present study. Allele sizes were determined using the GENESCAN 672 (version 1.2) and Genotyper (version 1.1) software (Applied Biosystems, Inc.) and converted into numbered alleles using the GAS program (version 2.1) (28).

Preliminary data analysis

Immunoglobulin levels for the whole sample were analysed using the SPSS statistical package for UNIX, version OSF/1 (SPSS Inc., IL). A natural logarithm transformation was used for IgM data; a square root transformation was used for the IgA and IgG data. Different transforming functions were assessed using normal probability and detrended plots to detect deviations from normality. Linear regression was used to examine the effects of smoking and age in each family group (fathers, mothers, sons and daughters). Immunoglobulin values were adjusted for the effects of significant covariates using linear regression followed by standardization in each family group.

Familial correlations

Familial correlations for adjusted immunoglobulin levels from the whole sample were examined using the REGC program from the S.A.G.E. package (29). Class D regressive models (30,31) specifying no major gene, Hardy–Weinberg proportions and no parent to offspring transmission (completely homogeneous) were used. Different patterns of familial correlations were tested using likelihood ratios.

Heritability calculations

Estimates of genetic heritability for IgA, IgG and IgM levels in the whole sample were calculated from the coefficients of regression of offsprings' phenotype on fathers' phenotype (32). A mean offspring phenotype was used for each nuclear family. Transformed but unadjusted phenotypes were used.

Linkage analysis

Sib-pair analysis of adjusted immunoglobulin levels was performed using the method of Haseman and Elston (33) implemented in the SIBPAL program from the S.A.G.E. package (29). This method detects linkage between a quantitative trait and a marker by regressing the square of the difference in sibs' trait values on the number of marker alleles identical by descent (2, 1 and 0). The regression coefficient divided by its standard error gives the test statistic for linkage, the significance of which can be determined. SIBPAL also provides a correction for multiple sibships and non-independence between sib-pairs. Possible linkage to the X chromosome was investigated by reclassifying each sib as affected or unaffected for high immunoglobulin levels. 'High' was defined as an immunoglobulin level greater than the 70th percentile of the age- and sex-matched population. Dichotomous (affected) sib-pair analysis was then carried out using a Hodge-weighting for multiple sibships (34) implemented in the GAS program (28).

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